JPRS: 19,287

OTS: 63-21868

20 May 1963

AD No. 406969
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THE ULTRAVIOLET FLUORESCENCE OF MUSCLES

[Following is a translation of an article by I. Ya. Barskiy, V.A. and Ye, M. Brumberg in the Russian-language journal Doklady Akademii Nauk SSSR (Reports of the Acad Sci USSR), Vol CXLVII, No 2, Moscow, 1963, pages 474-476.]

(Presented by Academician A. N. Terenin, 7 May 1962)

In studying the ultraviolet fluorescence of tissues of various organs of animals on histological slides, we have always noted a special fluorescence of muscle tissue [1, 2]. The maximum of energy radiation in the fluorescence spectrum of muscles is approximately 335-345 micromicrons; such a fluorescence is induced by short-wave ultraviolet rays of a wavelength of 320 micromicrons and less. A photometric comparison of the intensity of proper ultraviolet fluorescence of various tissues, for example on the tongue slides of a frog, showed that the striated muscles fluoresce eight times more intensively than connective tissue and four times more intensively than epithelial tissue [2]. The ultraviolet fluorescence of muscles designed for strenuous work is more intensive than fluorescence of muscles working with a lesser load.

Our experiments aimed at elucidating the nature of this fluorescence. It is a known fact that proteins containing aromatic aminoacids fluoresce in an ultraviolet field; the maximum energy in their fluorescence spectra, which is within the range of 330-340 micromicrons, is determined by the fluorescence of triptophan [3, 4]. It was of interest to compare the intensity of u-v fluorescence of various tissues with their content of tryptophan. This study was carried out by means of the histochemical method (Erlich's reaction) directly on slides of a number of organs

of animals, where one could have at once in one microscopic field section-areas of various tissues, as well as by means of determination of the total content of tryptophan in the slices of various tissues isolated from the organs and, as much as possible, of a homogenous nature (the new color reaction with n-toluol-sulfoacid [5]. It is virtually impossible to isolate for quantitative tests any given tissue without the admixture of others, as for instance muscle tissue, without the traces of connective tissue. We only tried to reduce to a minimum the admixture of another tissue to the tested one. This seemed to be satisfactory, since in this case we were interested in the approximate determination of the tryptophan content in the tissue with an accuracy of not higher than 20-30 percent. The qualitative histochemical tests showed that muscles contain only a slightly higher amount of tryptophan than epithelial and connective tissues. Similar results were obtained earlier by N. I. Yushkevich and B. V. Kedrovskiy [6]. A quantitative determination of the tryptophan content in various tissues by the above-mentioned method brought (with the accuracy of 20-30%) the following results: the concentration of tryptophan in the muscular, epithelial, and connective tissues is in proportion of 1.5: 1: 0.8.

It has been thus established that in the tested tissues no direct relationship is observed between the content of tryptophan and the intensity of ultraviolet fluorescence. This meant that the ultraviolet fluorescence of the muscles is apparently determined not by the presence of tryptophan-containing proteins, but to a greater extent by some other fluorescent substances or substance or something less probable, by tryptophan present in the muscles in some other state than it is in epithelial and connective tissues. A certain light on this matter could be thrown if, by means of microphotography of the fine muscular structure under ultraviolet fluorescence, an observation could be made under large magnification. We selected for this purpose the flying muscle of a fly in which one could distinguish separately under a microscope relatively large mitochondria -- sarcosomes (diameter: two to four microns) and motor fibrils, consisting of proteins with a relatively large content of tryptophan -- actin and myosin. The experiments were conducted on a live, or on a surviving muscle; for this purpose, a fine muscle fiber, obtained from the flying muscle, was placed on a microscope stand between a quartz slide and cover glass in a physiological solution used for insects. Such muscles are easily damaged; even if they are slightly torn or damaged

with a dissecting needle, some of the mitochondria "spill out" and float freely in the physiological solution.

A microphoto is cited of the fibers of the flying muscle of a fly obtained under the rays of ultraviolet fluorescence of a wavelength of 320-380 micromicrones. The muscle was slightly impaired in the process of preparation; therefore, in some sections the mitochondria were lost and only myofibrils, free of mitochondria, were visible. The photos were taken with a 58 x 0.80, quartz-fluorite achromatic lens, at aqueous immersion.(*)

The microphoto shows that the fluorescence of muscle mitochondria apparently constitutes the main part in the total fluorescence of muscles and that the protein fibrils fluoresce to a lesser extent. In view of the fact that mitochondria contain a relatively small quantity of tryptophan-containing proteins (at any rate, much less than the muscle myofibrils), we must conclude, in accordance with the above-described chemical tests, that the intensive ultraviolet fluorescence of the motor muscles is determined not by the cyclic aminoacides which enter the protein composition, but mainly by some other cyclic compounds, present in the mitochondria and apparently representing some prosthetic groups of the enzymic systems.

Earlier, in their experiments with male sexual cells of the acrididae (Stenobothrus sp.), N. A. Chernogryadskaya and M. S. Shudel' [8] showed that the ultraviolet fluorescence of these cells is concentrated in the mitochondria. This applies apparently also to many other cells. Our experiments on muscle cells fully confirm this conclusion. The authors [8] admit the possibility that the ultraviolet fluorescence of mitochondria belongs to phosphopyridinnucleotides (DPN and TPN). This hypothesis needs a thorough verification, since in the fluorescence of mitochondria also other cyclic compounds, present in the enzymic systems, can take part. In particular, they can be quinones in a deoxidated form (for example, coenzyme Q), since hydroquinone, as is known, fluoresces with a good yield, and the maximum of its fluorescence spectrum is located in the ultraviolet band within the range of 350-360 micromicrons. This problem is now investigated by the above-mentioned authors and in our

(*) For more details on the technique of photography with a ultraviolet fluorescent microscope see [1, 7].

laboratory by studying the effect of various influences and metabolic poisons on the cellular fluorescence, and the study of the visual characteristics of a number of substances present in the mitochondria. In particular, we recently demonstrated that DPN, even at room temperature, possesses together with fluorescence also a noticeable long-wave phosphorescence of a characteristic radiation spectrum with a pronounced maximum range of λ 505-510 micromicrones.

We pointed out in our previous works that ultraviolet fluorescence of cells often reflects their functional state. Apparently, in many instances this is connected with the fluctuation in the quantity of mitochondria and the intensity of the oxidation-reduction processes which are taking place in them. Presumably, upon further study, it will be possible to evaluate the amount or the state of mitochondria in cells and tissues, muscle tissue in particular, according to the intensity of the fluorescence of living, or appropriately processed, cells and tissues. These considerations, of course, could not be arbitrarily applied to any cellular fluorescence, since not only mitochondria may fluoresce in the cells, but also other structures of the cytoplasm or nucleus.

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Received 21 April 1962

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